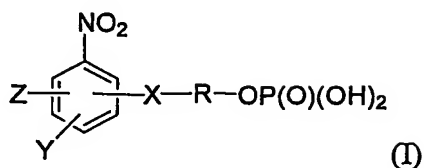


# Claims

1. A phosphate compound of Formula (I)



wherein:

X represents at any available ring position -CONH-, -SO<sub>2</sub>NH-, -O-, -CH<sub>2</sub>-, -NHCO- or -NHSO<sub>2</sub>-;

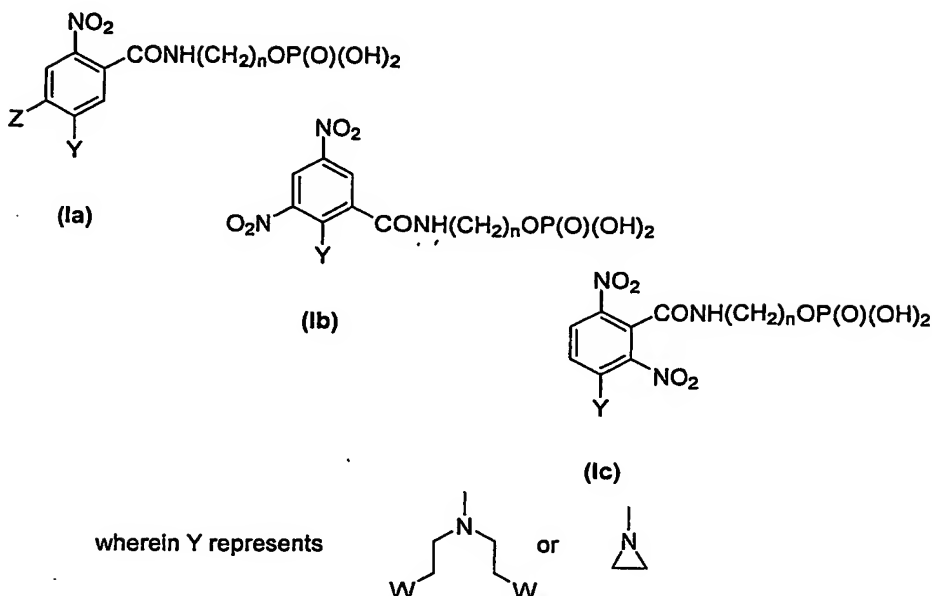
- 10 R represents a lower C<sub>1-6</sub> alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom;

Y represents at any available ring position -N-aziridinyl, -N(CH<sub>2</sub>CH<sub>2</sub>W)<sub>2</sub> or -N(CH<sub>2</sub>CHMeW)<sub>2</sub>, where each W is independently selected from halogen or -OSO<sub>2</sub>Me.

- 15 Z represents at any available ring position -NO<sub>2</sub>, -halogen, -CN, -CF<sub>3</sub> or -SO<sub>2</sub>Me;

and pharmaceutically acceptable salts and derivatives thereof.

2. A phosphate compound of Formula (I) as claimed in claim 1 which is selected  
20 from a compound represented by formulae (Ia), (Ib) or (Ic)



and wherein

n represents 1 to 6

5 Z represents -NO<sub>2</sub>, -halogen, -CN, -CF<sub>3</sub> or -SO<sub>2</sub>Me; and

where each W is independently selected from halogen or -OSO<sub>2</sub>Me

and pharmaceutically acceptable salts and derivatives thereof.

3. The phosphate compound of Formula (I) as claimed in claim 1 or claim 2 which is  
10 selected from:

2-[[2-[Bis(2-bromoethyl)amino]-3,5-dinitrobenzoyl]amino]ethyl dihydrogen phosphate;

3-[[5-[Bis(2-chloroethyl)amino]-2,4-dinitrobenzoyl]amino]propyl dihydrogen phosphate;

3-[[5-[Bis(2-bromoethyl)amino]-2,4-dinitrobenzoyl]amino]propyl dihydrogen phosphate;

2-[[2-[Bis(2-chloroethyl)amino]-3,5-dinitrobenzoyl]amino]ethyl dihydrogen phosphate;

15 2-[(2-Chloroethyl)-2,4-dinitro-6-[[[2-(phosphonooxy)ethyl]amino]-carbonyl]anilino]ethyl  
methanesulfonate;

2-({2-[Bis(2-bromopropyl)amino]-3,5-dinitrobenzoyl}amino)ethyl dihydrogen phosphate;

2-[(2-Bromoethyl)-2,4-dinitro-6-[[[2-(phosphonooxy)ethyl]amino]-carbonyl]anilino]ethyl  
methanesulfonate;

20 2-[[2-[Bis(2-iodoethyl)amino]-3,5-dinitrobenzoyl]amino]ethyl dihydrogen phosphate ;

2-[(2-Iodoethyl)-2,4-dinitro-6-({[2-(phosphonooxy)ethyl]amino}carbonyl)-anilino]ethyl  
methanesulfonate;

2-[(2-Chloroethyl)-2,4-dinitro-3-[[[3-(phosphonooxy)propyl]amino]-  
carbonyl]anilino]ethyl methanesulfonate;

3-({3-[Bis(2-bromoethyl)amino]-2,6-dinitrobenzoyl} amino)propyl dihydrogen phosphate;

2-[(2-Bromoethyl)-2,4-dinitro-3-[[[2-(phosphonooxy)ethyl]amino]-carbonyl]anilino]ethyl

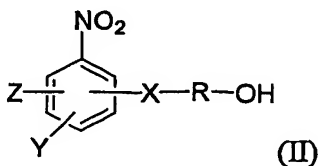
5 methanesulfonate;

2-[(2-Bromoethyl)-2,4-dinitro-3-[[[3-(phosphonooxy)propyl]amino]-  
carbonyl]anilino]ethyl methanesulfonate; and

2-[(2-Iodoethyl)-2,4-dinitro-3-[[[3-(phosphonooxy)propyl]amino]-carbonyl]anilino]ethyl  
methanesulfonate.

10

4. An alcohol compound of Formula (II)



wherein:

15 X represents at any available ring position -CONH-, -SO<sub>2</sub>NH-, -O-, -CH<sub>2</sub>-, -NHCO- or  
-NHSO<sub>2</sub>-;

Y represents at any available ring position -N-aziridiny, -N(CH<sub>2</sub>CH<sub>2</sub>W)<sub>2</sub>, or  
-N(CH<sub>2</sub>CH MeW)<sub>2</sub> where each W is independently selected from halogen or -OSO<sub>2</sub>Me;

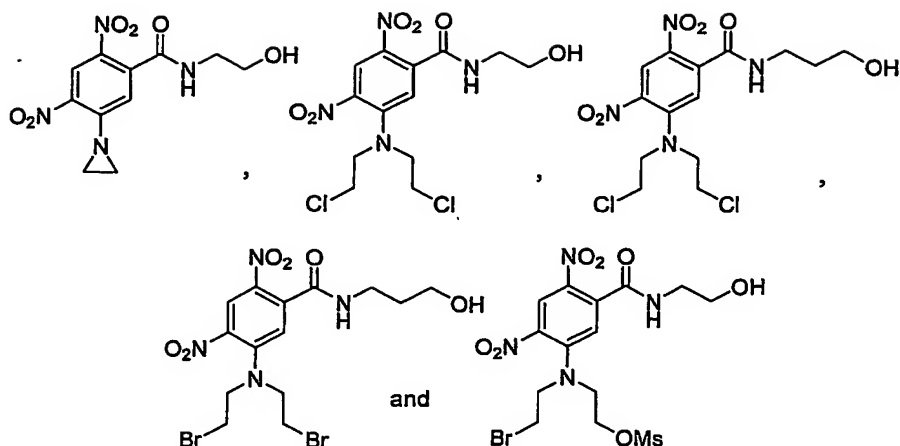
20

Z represents at any available ring position -NO<sub>2</sub>, -halogen, -CN, -CF<sub>3</sub> or -SO<sub>2</sub>Me;

R represents a lower C<sub>1-6</sub> alkyl optionally substituted with one or more groups including  
hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom; and

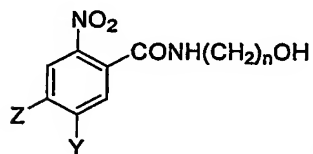
25 pharmaceutically acceptable salts and derivatives thereof, with the proviso that

69

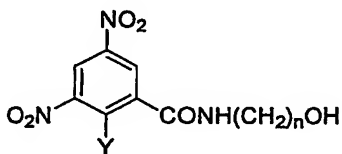


are excluded.

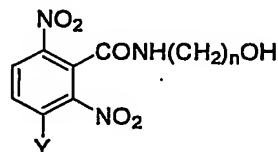
5. The alcohol compound of Formula (II) as claimed in claim 4 selected from a compound represented by formulae (IIa), (IIb) or (IIc)



(IIa)

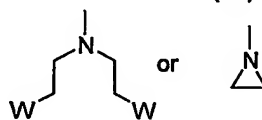


(IIb)



(IIc)

wherein Y may represent



and wherein

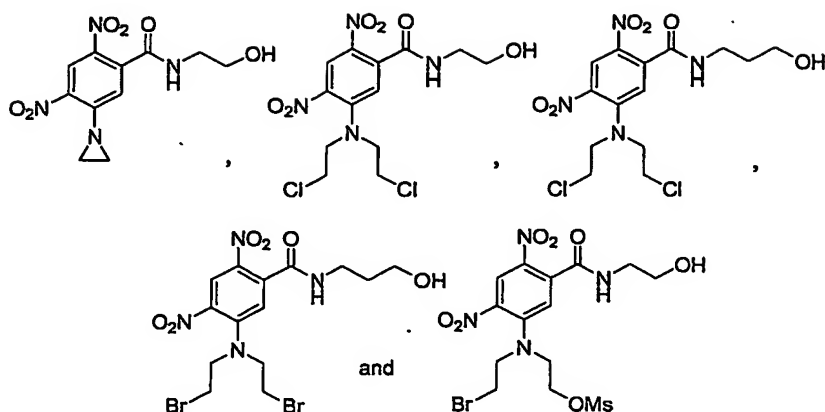
- 10 n represents 1 to 6

Z represents -NO<sub>2</sub>, -halogen, -CN, -CF<sub>3</sub> or -SO<sub>2</sub>Me; and

where each W is independently selected from halogen or -OSO<sub>2</sub>Me

and pharmaceutically acceptable salts and derivatives thereof with the proviso that

70



are excluded.

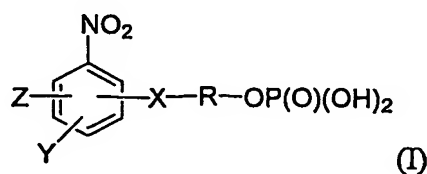
6. The alcohol compound of Formula (II) as defined in claim 5 selected from a  
5 compound of Formula (IIb) or (IIc) as defined in claim 5.

7. The alcohol compound of Formula (II) as defined in claim 5 or claim 6 selected  
from:

- N-(3-Hydroxypropyl)-5-[bis(2-chloroethyl)amino]-2,4-dinitrobenzamide;
- 10 N-(3-Hydroxypropyl)-5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzamide;
- N-(2-Hydroxyethyl)-5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzamide;
- N-(4-Hydroxybutyl)-5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzamide;
- N-(5-Hydroxypentyl)-5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzamide;
- N-(6-Hydroxyhexyl)-5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzamide;
- 15 5-[Bis(2-bromoethyl)amino]-N-(2-hydroxyethyl)-4-(methanesulfonyl)-2-nitrobenzamide;
- 2[(2-Bromoethyl)-5-[(3-hydroxypropyl)amino]carbonyl]-2,4-dinitroanilino]ethyl  
methanesulfonate;
- 5-[Bis(2-iodoethyl)amino]-N-(2-hydroxyethyl)-2,4-dinitrobenzamide;
- 2-[Bis(2-Chloroethyl)amino]-N-(2-hydroxyethyl)-3,5-dinitrobenzamide;
- 20 2-[Bis(2-bromoethyl)amino]-N-(2-hydroxyethyl)-3,5-dinitrobenzamide;
- 2-[Bis(2-chloroethyl)amino]-N-(3-hydroxypropyl)-3,5-dinitrobenzamide;
- 2-[Bis(2-bromoethyl)amino]-N-(3-hydroxypropyl)-3,5-dinitrobenzamide;
- 2-[Bis(2-chloroethyl)amino]-N-(4-hydroxybutyl)-3,5-dinitrobenzamide;
- 2-[Bis(2-bromoethyl)amino]-N-(4-hydroxybutyl)-3,5-dinitrobenzamide;
- 25 2-[Bis(2-chloroethyl)amino]-N-(5-hydroxypentyl)-3,5-dinitrobenzamide;

- 2-[Bis(2-bromoethyl)amino]-N-(5-hydroxypentyl)-3,5-dinitrobenzamide;  
 2-[Bis(2-chloroethyl)amino]-N-(6-hydroxyhexyl)-3,5-dinitrobenzamide;  
 2-[Bis(2-bromoethyl)amino]-N-(6-hydroxyhexyl)-3,5-dinitrobenzamide;  
 2-[Bis(2-bromopropyl)amino]-N-(2-hydroxyethyl)-3,5-dinitrobenzamide;  
 5 2-((2-Bromoethyl)-2-{[(2-hydroxypropyl)amino]carbonyl}-4,6-dinitroanilino)ethyl  
 methanesulfonate;  
 2-((2-Bromoethyl)-2-{[(2-hydroxyethyl)amino]carbonyl}-4,6-dinitroanilino)ethyl  
 methanesulfonate;  
 2-((2-Chloroethyl)-2-{[(2-hydroxyethyl)amino]carbonyl}-4,6-dinitroanilino)ethyl  
 10 methanesulfonate;  
 2-[Bis(2-iodoethyl)amino]-N-(2-hydroxyethyl)-3,5-dinitrobenzamide;  
 2-((2-Iodoethyl)-2-{[(2-hydroxyethyl)amino]carbonyl}-4,6-dinitroanilino)ethyl  
 methanesulfonate;  
 3-[Bis(2-bromoethyl)amino]-N-(2-hydroxyethyl)-2,6-dinitrobenzamide;  
 15 2-((2-Bromoethyl)-3-{[(2-hydroxyethyl)amino]carbonyl}-2,4-dinitroanilino)ethyl  
 methanesulfonate;  
 3-[Bis(2-bromoethyl)amino]-N-(3-hydroxypropyl)-2,6-dinitrobenzamide;  
 2-((2-bromoethyl)-3-{[(3-hydroxypropyl)amino]carbonyl}-2,4-dinitroanilino)ethyl  
 methanesulfonate;  
 20 3-[Bis(2-bromoethyl)amino]-N-(4-hydroxybutyl)-2,6-dinitrobenzamide;  
 2-((2-Bromoethyl)-3-{[(4-hydroxybutyl)amino]carbonyl}-2,4-dinitroanilino)ethyl  
 methanesulfonate;  
 2-((2-Chloroethyl)-3-{[(3-hydroxypropyl)amino]carbonyl}-2,4-dinitroanilino)ethyl  
 methanesulfonate; and  
 25 2-((2-Iodoethyl)-3-{[(3-hydroxypropyl)amino]carbonyl}-2,4-dinitroanilino)ethyl  
 methanesulfonate.

8. A method of preparing a phosphate represented by the general formula (I);



wherein:

X represents at any available ring position -CONH-, -SO<sub>2</sub>NH-, -O-, -CH<sub>2</sub>-, -NHCO- or -NHSO<sub>2</sub>-;

5

R represents a lower C<sub>1-6</sub> alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom;

Y represents at any available ring position -N-aziridinyll or -N(CH<sub>2</sub>CH<sub>2</sub>W)<sub>2</sub>, where each W is independently selected from halogen or -OSO<sub>2</sub>Me;

10

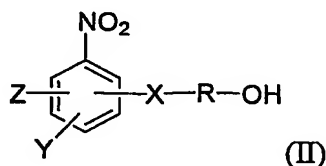
Z represents at any available ring position -NO<sub>2</sub>, -halogen, -CN, -CF<sub>3</sub> or -SO<sub>2</sub>Me;

and pharmaceutically acceptable salts and derivatives thereof;

the method including the step of

15

(i) phosphorylating a compound of formula (II)



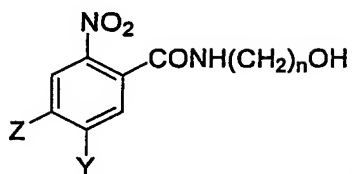
wherein:

X represents at any available ring position -CONH-, -SO<sub>2</sub>NH-, -O-, -CH<sub>2</sub>-, -NHCO- or  
20 -NHSO<sub>2</sub>-;

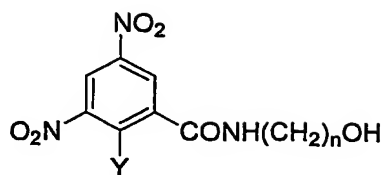
Y represents at any available ring position -N-aziridinyll, -N(CH<sub>2</sub>CH<sub>2</sub>W)<sub>2</sub>, or -N(CH<sub>2</sub>CH MeW)<sub>2</sub> where each W is independently selected from halogen or -OSO<sub>2</sub>Me;

25 Z represents at any available ring position -NO<sub>2</sub>, -halogen, -CN, -CF<sub>3</sub> or -SO<sub>2</sub>Me; and R represents a lower C<sub>1-6</sub> alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom.

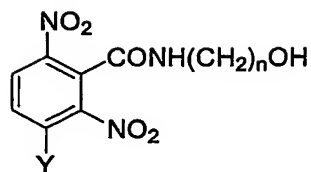
9. A method of preparing a compound of formulae (IIa), (IIb) or (IIc)



(IIa)

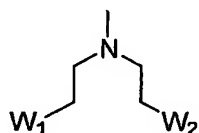


(IIb)



(IIc)

wherein Y may represent



and wherein

n represents 1 to 6

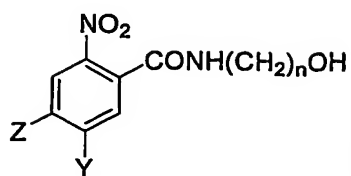
5 Z represents -NO<sub>2</sub>, -halogen, -CN, -CF<sub>3</sub> or -SO<sub>2</sub>Me; and

where W<sub>1</sub> is halogen and W<sub>2</sub> is -OSO<sub>2</sub>Me

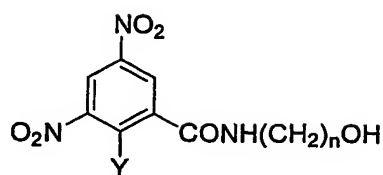
and pharmaceutically acceptable salts and derivatives thereof;

the method including the step of

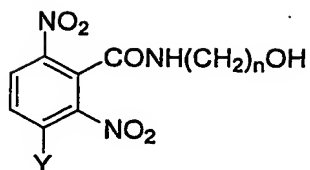
10 reacting a compound of formulae (IIa'), (IIb') or (IIc') optionally with heating



(IIa')

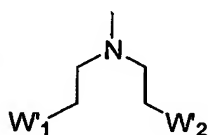


(IIb')



(IIc')

wherein Y may represent



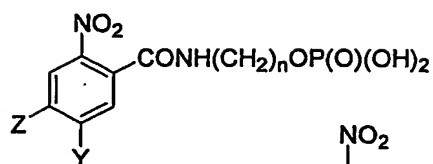
wherein  $W'_1$  and  $W'_2$  are each halogen;

with an effective amount of silver methanesulfonate (AgOMs) in a solvent to give a  
 5 compound of formulae (IIa), (IIb) or (IIc) defined above in this claim.

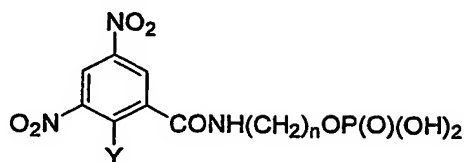
10. The method as claimed in claim 9 wherein the solvent is selected from MeCN or other polar non-protic solvent.

10 11. A method of preparing a compound of formulae (Ia), (Ib) or (Ic)

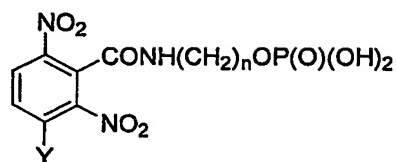
75



(Ia)

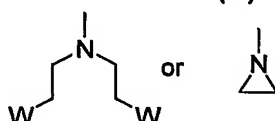


(Ib)



(Ic)

wherein Y may represent



and wherein

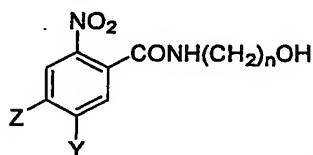
n represents 1 to 6

Z represents -NO<sub>2</sub>, -halogen, -CN, -CF<sub>3</sub> or -SO<sub>2</sub>Me; and

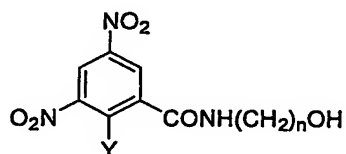
- 5 where each W is independently selected from halogen or -OSO<sub>2</sub>Me  
and pharmaceutically acceptable salts and derivatives thereof

the method including the step of

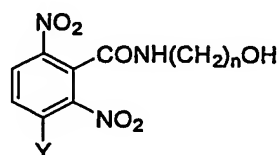
phosphorylating a compound represented by formulae (IIa), (IIb) or (IIc)



(IIa)

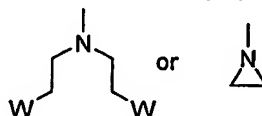


(IIb)



(IIc)

wherein Y represents



and wherein

n represents 1 to 6

Z represents -NO<sub>2</sub>, -halogen, -CN, -CF<sub>3</sub> or -SO<sub>2</sub>Me; and

where each W is independently selected from halogen or -OSO<sub>2</sub>Me

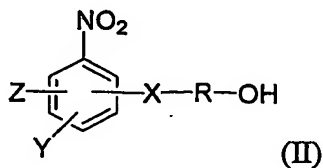
5 and pharmaceutically acceptable salts and derivatives.

12. A compound of formula (I) when obtained by the method defined in claim 8.

13. A compound of formula (Ia), (Ib) or (Ic) when obtained by the method defined in  
10 claim 11.

14. A compound of formula (IIa), (IIb) or (IIc) obtained by the method defined in claim 9 or claim 10.

15. A method of cell ablation utilising at least one nitroreductase enzyme including the step of administering a compound of Formula (I) as defined above in any one of claims 1 to 3 or a compound of Formula (II)



wherein:

20 X represents at any available ring position -CONH-, -SO<sub>2</sub>NH-, -O-, -CH<sub>2</sub>-, -NHCO- or -NHSO<sub>2</sub>-;

Y represents at any available ring position -N-aziridiny, -N(CH<sub>2</sub>CH<sub>2</sub>W)<sub>2</sub>, or -N(CH<sub>2</sub>CH MeW)<sub>2</sub> where each W is independently selected from halogen or -OSO<sub>2</sub>Me;

25

Z represents at any available ring position -NO<sub>2</sub>, -halogen, -CN, -CF<sub>3</sub> or -SO<sub>2</sub>Me;

R represents a lower C<sub>1-6</sub> alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom; and

30 pharmaceutically acceptable salts and derivatives thereof;

or a mixture thereof in an effective amount to ablate cells wherein said cells express at least one nitroreductase enzyme.

16. The method as claimed in claim 15 wherein the at least one nitroreductase  
5 enzyme is encoded for by the *nfsB* gene of either *E. coli* or by orthologous genes in *Clostridia* species.

17. The method as claimed in claim 15 or claim 16 wherein the cell ablation  
provides a substantially minimal bystander effect.

10

18. The method as claimed in any one of claims 15 to 17 wherein the cells that  
express the at least one nitroreductase enzyme are tumour cells in tissue in a subject.

19. The method as claimed in any one of claims 15 to 18 wherein the cell ablation  
15 is achieved through GDEPT (gene-directed enzyme-prodrug therapy).

20. The method as claimed in any one of claims 15 to 19 wherein the cell ablation  
is achieved through ADEPT (antibody-directed enzyme-prodrug therapy).

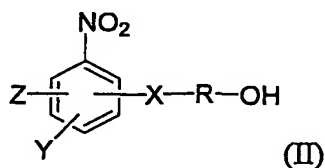
20 21. The method as claimed in any one of claims 15 to 20 wherein the cells are  
mammalian.

22. A method of providing anticancer therapy, wherein a compound of Formula (I)  
as defined above in any one of claims 1 to 3 is administered in a therapeutically  
25 effective amount to tumour cells in a subject.

23. The method as claimed in claim 22 wherein the therapeutically effective  
amount administered is between about 20% to 100% of the maximum tolerated dose  
of said subject.

30

24. The method as claimed in claim 21 or claim 22 wherein the compound of Formula (I) or Formula (II) is administered for use in cell ablation in conjunction with at least one nitroreductase enzyme.
25. The method as claimed in claim 24 wherein the cell ablation is achieved through GDEPT (gene-directed enzyme-prodrug therapy) or ADEPT (antibody-directed enzyme prodrug therapy).
26. The method as claimed in claim 24 or claim 25 wherein the at least one nitroreductase enzyme is encoded for by the *nfsB* gene of either *E. coli* or by orthologous genes in *Clostridia* species.
27. The method as claimed in any one of claims 21 to 26 including the further step of irradiating the tumour cells.
28. A pharmaceutical composition including a therapeutically effective amount of a compound of Formula (I) as defined in any one of claims 1 to 3 or a compound of Formula (II)

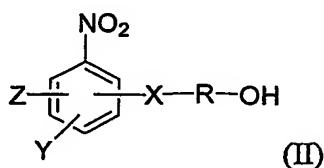


wherein:

- X represents at any available ring position -CONH-, -SO<sub>2</sub>NH-, -O-, -CH<sub>2</sub>-, -NHCO- or -NHSO<sub>2</sub>-;
- Y represents at any available ring position -N-aziridinyl, -N(CH<sub>2</sub>CH<sub>2</sub>W)<sub>2</sub> or -N(CH<sub>2</sub>CHMeW)<sub>2</sub>, where each W is independently selected from halogen or -OSO<sub>2</sub>Me;
- Z represents at any available ring position -NO<sub>2</sub>, -halogen, -CN, -CF<sub>3</sub> or -SO<sub>2</sub>Me;

R represents a lower C<sub>1-6</sub> alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom; and pharmaceutically acceptable salts and derivatives thereof, or a mixture thereof, and a pharmaceutically acceptable excipient, adjuvant, carrier, buffer  
 5 or stabiliser.

29. The use in the manufacture of a medicament of an effective amount of a compound of Formula (I) as defined in any one of claims 1 to 3 or a compound of Formula (II)



10 wherein:

X represents at any available ring position -CONH-, -SO<sub>2</sub>NH-, -O-, -CH<sub>2</sub>-, -NHCO- or -NHSO<sub>2</sub>-;

Y represents at any available ring position -N-aziridiny, -N(CH<sub>2</sub>CH<sub>2</sub>W)<sub>2</sub> or -N(CH<sub>2</sub>CH  
 15 MeW)<sub>2</sub>, where each W is independently selected from halogen or -OSO<sub>2</sub>Me;

Z represents at any available ring position -NO<sub>2</sub>, -halogen, -CN, -CF<sub>3</sub> or -SO<sub>2</sub>Me;

R represents a lower C<sub>1-6</sub> alkyl optionally substituted with one or more groups including  
 20 hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom; and pharmaceutically acceptable salts and derivatives thereof, or mixtures thereof, to ablate cells that express at least one nitroreductase enzyme.

30. The use as claimed in claim 29 wherein the medicament is further adapted for  
 25 GDEPT (gene-directed enzyme-prodrug therapy) or ADEPT (antibody-directed enzyme therapy).

31. The use as claimed in claim 29 or claim 30 wherein the at least one nitroreductase enzyme is encoded for by the nfsB gene of either *E. coli* or by  
 30 orthologous genes in *Clostridia* species.

32. The use as claimed in any one of claims 29 to 31 wherein the medicament is adapted to ablate target cells with a substantially minimal bystander effect.
- 5 33. The use as claimed in any one of claims 29 to 31 wherein the medicament is adapted for mammalian cells.